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**DECLARATION OF DR. AYOUB RASHTCHIAN UNDER 37 C.F.R. § 1.132**

I hereby declare the following:

1. I am Dr. Ayoub Rashtchian, a citizen of the United States.

2. I am the Founder and Chief Science Officer of Quanta Biosciences

3. I am one of the inventors of Patent Application Serial No.: 10/633,629 ("'629") and I am familiar with the subject matter of '629 and the related art.

4. I have read and understood the Office Action mail date May 2, 2007.

5. The appended document titled "Effect of Vortexing on qPCR" describes experiments performed under my direction to test the effect of vortexing, ie., bubbling, on qPCR. The results of these experiments are shown in the appended document "Effect of Vortexing on Detection of 10,000 copies of ACTB Using SYBR Green Qpcr.

6. The results show that there was no difference in the detection of ACTB for reaction mixtures subjected to vortexing for varying times relative to control samples that were not vortexed.

7. These data demonstrate that vortexing and the resulting formation of bubbles had no effect on the capacity for Taq DNA polymerase to amplify ACTB in a SYBR Green real-time PCR reaction.

8. In sum, the results show that foaming does not inactivate Taq polymerase.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment or both under §1001 of Title 18 of the United States Code and may jeopardize the validity or enforceability of the above-identified application or any patent issued thereon.

A. Rashtchian

Ayoub Rashtchian

11-02-07

Date

Effect of Vortexing on qPCR

To test the effect of vortexing on qPCR a single master mix containing all required components for SYBR Green qPCR of the ACTB gene was prepared as follows:

| | |
|---|---|
| Water | 702 µL |
| iQ SYBR Green Supermix (2X) | 750 µL |
| 10 µM (each) ACTB Primer Mix | 45 µL |
| ACTB plasmid DNA (1 x $10^5$ copies / µL) | 3 µL |
| Final Volume | 1500 µL |

The master mix was gently mixed by inversion on a rocker platform for 5 minutes at +4°C so as not to create bubbles.

Equal aliquots (300 µL) were transferred to separate 1.5-mL tubes sitting on ice. Each tube was vortexed at the maximum setting for varying lengths of time (t = 0, 15, 30, 60, or 120 seconds). Tubes were photographed following the different vortexing times to clearly document the formation of bubbles in these mixtures containing Taq DNA polymerase. The control tube (C) was not vortexed (t=0).

Quadruplicate qPCR reactions (50 µL) were carried out for each mixture. A multi-dispensing digital pipettor was used to remove a 200 µL sample from the bottom of each tube and 50-µL aliquots were then dispensed into a 96-well PCR reaction plate. Since bubbles can impart optical dispersions that affect the measurement of fluorescent signal in real-time PCR, care was exercised to avoid pipetting any of the bubbles or foam that resulted from vortexing.

The plate was sealed with an optically clear heat-seal film (Abgene) and then transferred to an iCycler iQ Real-Time PCR System (Bio-Rad Laboratories). Reactions were cycled using experimental plate well factors as follows: 1 cycle of 95°C for 3 min; 45 cycles of 95°C for 10 seconds, 62°C for 45 seconds. Data was collected and analyzed at the 62°C annealing/extension step.

Equivalent cycle threshold values were obtained for all samples. There was no difference in the detection of ACTB for reaction mixtures subjected to vortexing for varying times relative to control samples that were not vortexed. These data demonstrate that vortexing and the resulting formation of bubbles had no effect on the capacity for Taq DNA polymerase to amplify the ACTB in a SYBR Green real-time PCR reaction. Results are presented below.

Effect of antifoams on precision of qPCR:

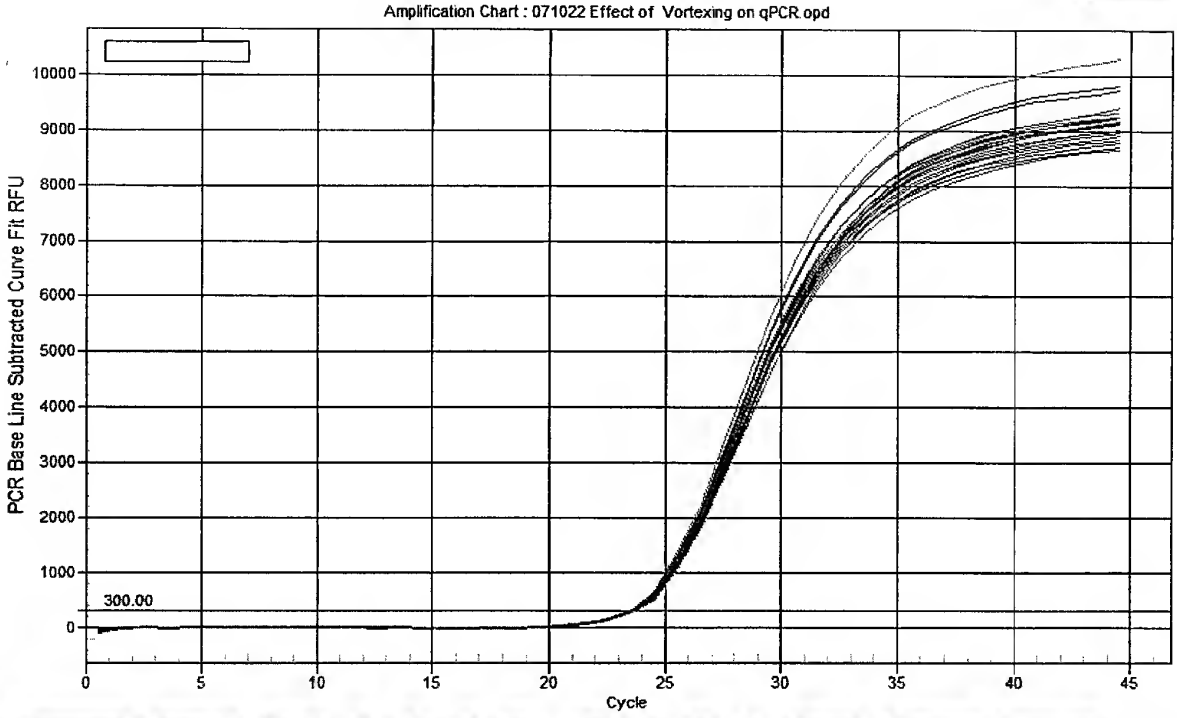
The present invention has demonstrated that inclusion of appropriate anti-foam agents in qPCR prevents formation of bubbles in qPCR reactions and provides qPCR measurements that are free of artifacts. This results in significantly more accurate qPCR results. As discussed in the introduction, qPCR is the method of choice for measurement of gene expression and often time is used as gold standard for confirmation of differential gene expression results obtained using other methodologies. Therefore it is critical that the gold standard method to be as accurate and reliable as possible.

The absolute accuracy and reliability of qPCR experimentation has long been felt by the scientific and diagnostic community. For example this technique is the only method by which the effectiveness of anti- Aids drugs can be tested on patients with Aids and accuracy of the viral load determination can have a significant effect on the decisions related to therapy. There are numerous web pages and documents published by others that warn users of qPCR to be aware of bubbles in their reactions. (see attached documents). Prior to our invention, no one has used antifoam agents to suppress bubbles in qPCR .

Comparison of the data presented in Figures 2 and 4 demonstrate the significance of precision in qPCR and the improvements provided by the methods of this invention. Figure 2 and Figure 4 demonstrate data generated from identical reactions containing 20 copies of template DNA per reaction. The only difference between the experiments is the inclusion of antifoam in reaction in Figure 4; while the reactions in Figure 2 are without antifoam and susceptible to bubble artifacts.
As it can be seen bubbles caused a significant error in wells H1 to H6 and the Ct values ranged from 33 to 38 for these wells with identical amount of target DNA. The variation in the Ct values ( 5 Ct's) represents approximately 15 fold difference in quantification error. On the other hand the greatest variation for reactions containing antifoam was 1 Ct which equals to 2 fold difference in quantification.

Effect of Vortexing on Detection of 10,000 copies of ACTB Using SYBR Green qPCR

| | Cycle Threshold (Ct) | | | | | |
|---|---|---|---|---|---|---|
| replicate no. | n1 | n2 | n3 | n4 | AVG Ct | Std. Dev. |
| Control | 23.71 | 23.65 | 23.54 | 23.61 | 23.63 | 0.07 |
| Vortex-15s | 23.68 | 23.52 | 23.65 | 23.68 | 23.63 | 0.08 |
| Vortex-30s | 23.52 | 23.34 | 23.45 | 23.60 | 23.48 | 0.11 |
| Vortex-60s | 23.53 | 23.43 | 23.34 | 23.36 | 23.42 | 0.09 |
| Vortex-120s | 23.62 | 23.51 | 23.60 | 23.54 | 23.57 | 0.05 |




Control No Vortexing | 15 sec | 30 sec | 1 min | 2 min | Vortex Time